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REVIEW AND SYNTHESIS

Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies

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Abstract

Nitrogen (N) enrichment is an element of global change that could influence the growth and abundance of many organisms. In this meta-analysis, I synthesized responses of microbial biomass to N additions in 82 published field studies. I hypothesized that the biomass of fungi, bacteria or the microbial community as a whole would be altered under N additions. I also predicted that changes in biomass would parallel changes in soil CO_2 emissions. Microbial biomass declined 15% on average under N fertilization, but fungi and bacteria were not significantly altered in studies that examined each group separately. Moreover, declines in abundance of microbes and fungi were more evident in studies of longer durations and with higher total amounts of N added. In addition, responses of microbial biomass to N fertilization were significantly correlated with responses of soil CO_2 emissions. There were no significant effects of biomes, fertilizer types, ambient N deposition rates or methods of measuring biomass. Altogether, these results suggest that N enrichment could reduce microbial biomass in many ecosystems, with corresponding declines in soil CO_2 emissions.

Keywords

Bacteria, belowground carbon allocation, biome, carbon dioxide, chloroform fumigation, fertilization, fungi, nitrogen deposition, nitrogen limitation.

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INTRODUCTION

Microbes control atmospheric concentrations of a number of important greenhouse gases (Conrad 1996). For example, bacteria (eubacteria + archaea) and fungi release CO_2 as a product of decomposition. In addition, bacteria are the primary producers of CH_4 and N_2O, with some additional production of N_2O by fungi (Shoun *et al.* 1992). Thus, the responses of each of these groups to environmental parameters may have considerable consequences for climate change.

Nitrogen (N) availability, for instance, is known to influence strongly the growth and abundance of organisms (Vitousek & Howarth 1991). This issue is germane because anthropogenic activities have more than doubled global rates of N fixation and deposition (Vitousek *et al.* 1997). Net primary productivity (NPP) of plants is N-limited worldwide, and NPP could increase by as much as 30% under N deposition (LeBauer & Treseder 2008). However, fungi and bacteria may not necessarily be limited by the same elements that limit plants (Hobbie 2005). Microbes could be limited by carbon (C), water or phosphorus instead (Waksman & Tenney 1927; Wardle 1992).

Indeed, N additions have been shown to negatively affect microbial growth in several field and laboratory studies (e.g. Soderstrom *et al.* 1983; Nohrstedt *et al.* 1989), which indicates that microbes are not always limited by N. A number of mechanisms have been proposed for the decline in fungal and bacterial biomass under N enrichment (Fig. 1) (Baath *et al.* 1981). For example, osmotic potentials in soil solution could become toxic owing to the introduction of additional ions via fertilizer (Broadbent 1965). In this way, N additions could directly inhibit microbial growth. Nitrogen saturation can also decrease soil pH, leading to leaching of magnesium and calcium and mobilization of aluminium (Vitousek *et al.* 1997). Microbes may become magnesium- or calcium-limited, or they may experience aluminium toxicity.

Other mechanisms could elicit indirect effects by altering the availability of C. For example, excess N can inhibit ligninase production by white rot fungi (Ander & Eriksson 1977; Keyser *et al.* 1978; Waldrop & Zak 2006). As lignin can bind to – or physically block access to – other compounds such as cellulose in plant tissues, its presence can hinder the ability of microbes to access these other compounds for C or energy (Swift *et al.* 1979; Fog 1988;

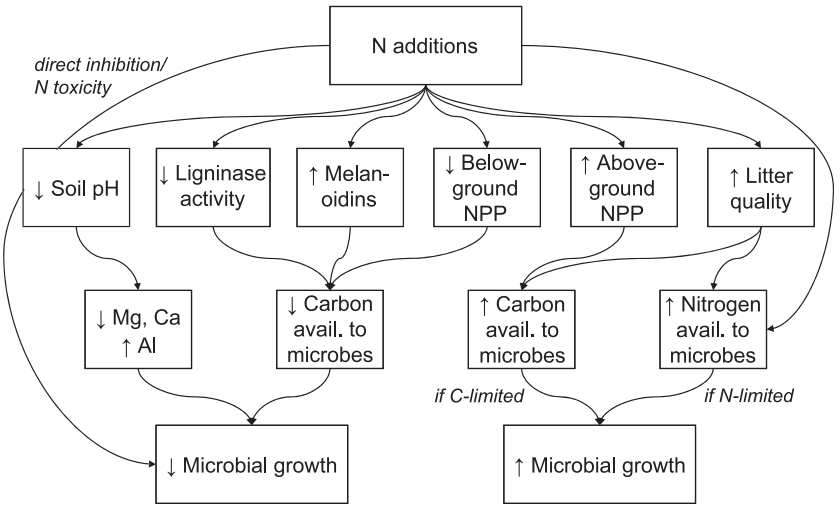

Figure 1 Potential mechanisms for N effects on microbial growth.

Sinsabaugh & Linkins 1989; Hammel 1997; Malherbe & Cloete 2002). Nitrogen fertilization can also reduce investment by plants in fine roots and mycorrhizal fungi, because these structures become less critical for maintaining nutrient uptake (Aerts & Chapin 2000; Treseder 2004). As a result, less C would be deposited in the soil via turnover of belowground tissues. In addition, nitrogenous compounds can condense with carbohydrates, resulting in the production of melanoidins (Soderstrom *et al.* 1983; Fog 1988), and they can increase the polymerization of polyphenols into 'brown' compounds (Haider & Martin 1967). Both products are relatively recalcitrant to decomposition. These processes could accentuate C limitation of soil microbes.

On the other hand, aboveground litter production usually increases following N fertilization (LeBauer & Treseder 2008), which could alleviate C limitation as litter becomes incorporated into soil. This mechanism could be especially important in the uppermost layers of soil. Plant community composition also frequently shifts, often towards species with greater litter quality (Chapin *et al.* 1986; Stevens *et al.* 2004; Suding *et al.* 2005). The proportion of labile C compounds within plant litter could increase as a result. These mechanisms, in addition to alleviation of microbial N-limitation, may be responsible for any observed increases in microbial biomass.

Although effects of N fertilization on microbes have been assessed in numerous field studies, inconsistencies among results have hampered our capacity to draw general conclusions. To improve our ability to predict changes in microbial abundance under anthropogenic N deposition – and consequences for production of greenhouse gases – I conducted a meta-analysis of published responses to N. I hypothesized that N additions would alter the biomass of microbes in ecosystems via one or more mechanisms included in Fig. 1. Furthermore, I predicted that N effects would scale proportionately with the annual rate of N fertilization, with the total amount of N added over time ('N load'), and with the duration of N fertilization. Moreover, I expected that background N deposition would accentuate any declines in microbial biomass (by inducing greater N toxicity or C limitation), and would mitigate any increases in biomass (by reducing baseline N limitation of microbes or plants). I also hypothesized that changes in microbial biomass should result in concurrent changes in soil CO_2 release. In addition, I examined whether results were consistent among biomes and fertilizer types. Where possible, these questions were tested separately for studies that targeted fungi, bacteria and the microbial community as a whole (fungi + bacteria). These groups could vary in their responses to N, and each has different controls over atmospheric trace gases.

METHODS

Sources of data

Meta-analyses were performed on previously published data that met specific criteria (see Appendix S1 in Supporting Information), following Treseder (2004). In particular, I focused on field studies in which microbial biomass was measured in response to long-term (> 1 month), plot-scale (> 0.8 m^2) enrichment in N availability, in comparison with an unenriched control. To avoid confounding effects of C or phosphorus enrichment, I excluded studies that added organic N or urea, or in which phosphorus fertilizer was applied in combination with N. Furthermore, because one of the objectives of this meta-analysis was to better predict microbial responses to N in ecosystems, microcosm or greenhouse-based experiments were not considered. In addition, I limited my data collection to results in which means, standard deviations (SDs) and replicate numbers were reported or could be determined.

Because one assumption of meta-analysis is that studies are independent from one another (Gurevitch & Hedges 1999), I primarily used only one set of data from a given system. For instance, microbial biomass was often measured several times within a given study, in which case I restricted my analyses to the latest sampling date (except for the test of effects of study duration). If more than one publication presented results from the same field plots, I relied upon data from the most recent or most comprehensive paper. Where soils were sampled at multiple depths, I used data from the uppermost layer. In addition, several studies applied N at a range of levels; in these cases, I only included data associated with the highest application rates (except when effects of N fertilization rates or N loads were examined). Conversely, if a particular publication reported results from more than one study system that could reasonably be considered independent (e.g. geographical location, ecosystem, dominant vegetation type or fertilizer type), each system was designated as a different study.

Data acquisition

For each study, meta-analysis requires the mean, SD and replicate number (n) for the control as well as the N-addition treatment. When means and errors were presented in a graph, I digitized the data by using Engauge Digitizer 4.1 (http://digitizer.sourceforge.net). If standard errors (SEs) were reported, these were transformed according to the equation: SE = SD ($n^{-1/2}$). Unidentified error bars were assumed to represent SE.

Biomass measurements

Authors used several methods to assess biomass (Appendix S1). Microbial (bacterial + fungal) biomass was determined via chloroform fumigation extraction or incubation for microbial C (Brookes *et al.* 1985), total amounts of phospholipid fatty acid (PLFA) in soil (Frostegard & Baath 1996) or abundance of ATP in soil (Eiland 1983). To measure fungal biomass, investigators used microscopy-based counts of soil extracts, PLFAs specific to fungi and minirhizotron images collected *in situ* (Tingey *et al.* 1997). For bacteria, microscopy, bacteria-specific PLFAs, quantitative PCR with universal bacterial primers (Fierer *et al.* 2005) and concentrations of muramic acid in soils (Vishnevetsky & Steinberger 1996) were assessed. I did not consider studies in which microbial biomass was estimated based on response to non-N compounds such as C or enriched media. Thus, measurements of substrate-induced respiration, most probable number counts and BIOLOG (BIOLOG, Inc., Hayward, CA, USA) assays of substrate use were not included. Moreover, studies that focused on specific groups of fungi or bacteria (e.g. arbuscular mycorrhizal fungi, ammonia oxidizers, methanotrophs, or nitrogen-fixers) were omitted to avoid biasing results towards subsets of the fungal or bacterial communities.

Background N deposition

I used the 'Nitrogen deposition onto the United States and Western Europe' dataset (Holland *et al.* 2005) to estimate background rates of wet N deposition for applicable studies. This dataset includes 0.5 × 0.5 degree resolution maps of wet NH_4^+ and NO_3^- deposition, as interpolated from a network of *in situ* measurements collected in 1978–1994. I was able to include North American or Western European studies for which latitude and longitude, or sufficient information to determine latitude and longitude, were reported. For each of these sites, I summed the wet deposition rates for NH_4^+ and NO_3^- that were reported for the corresponding latitude/longitude grid cell (Appendix S1).

Soil CO_2 emissions

Where soil CO_2 emissions were reported, authors had obtained them via laboratory incubations of soil, soil chambers in field sites, and measurements of CO_2 release from freshly collected and sieved soil samples (Appendix S1).

Statistics

Meta-analyses were used to determine the significance of biomass responses to N enrichment. For each study and each target group (fungi, bacteria or microbes), the effect size was calculated as the natural log of the response ratio ('R'), which is the mean of the treatment divided by the mean of the control. An R of 1 indicates that the N addition had no effect. The estimate of variance for each study was represented as $v_{\ln R}$, which is based on means, SDs, and replicate numbers for controls and treatments (Hedges *et al.* 1999).

To determine if N additions had a significant effect, I applied a random effects model using MetaWin software (Rosenberg *et al.* 2000). Bias-corrected bootstrap 95% confidence intervals (CIs) were calculated for each mean R. If the 95% CIs of R did not overlap with 1, then responses were significant at $P < 0.05$. Moreover, random effects models allow comparisons among groups in a framework similar to ANOVA. I sequentially compared responses among biomes, fertilizer type and measurement type. I also used continuous model meta-analyses to test for relationships between r and annual rates of N fertilization, ambient wet N deposition, N load and duration of fertilization. In addition, I used continuous models to test for the effects of plot size and replicate number; in these cases, data from fungi, bacteria and microbe studies were combined. Statistical results reported include R; 95% CIs for

R (CI); total heterogeneity in *R* among studies (Q_T); and in the case of comparisons among groups, the difference among group cumulative effect sizes (Q_M), and the residual error (Q_E) (Rosenberg *et al.* 2000). I conducted a Kendall's tau rank correlation test (Sokal & Rohlf 1995) to test for the relationship between replicate number of each study and standardized effect size (Rosenberg *et al.* 2000). Such a relationship would indicate a publication bias in which larger N effects were more likely to be published than were smaller N effects. Finally, I applied a Pearson correlation to analyse the relationship between *R* of biomass and *R* of soil CO_2 respiration for studies in which both were reported. As a limited number of studies reported both variables, results from all three target groups were combined.

RESULTS

Microbes

Microbial biomass declined significantly under N fertilization, by an average of 15.4% across studies (Table 1). Responses were somewhat inconsistent among studies, as indicated by a marginally significant Q_T value ($Q_T = 40.71$, $P = 0.057$). As N load increased, effects on microbial biomass became significantly more negative (Fig. 2). Two studies had particularly high N loads of 4032 and 5185 kg N m^{-2} (McAndrew & Malhi 1992; Deng *et al.* 2006); when they were excluded, the regression was still significant ($\ln R = -0.0001 * \text{N load} - 0.02$, $P = 0.035$). In addition, longer durations of N fertilization elicited stronger declines in microbial abundance, especially within the first 5 years (Fig. 2). When the two longest studies (Collins *et al.* 1992) were omitted from the analysis, duration effects remained significant ($R = -0.154 * \ln[\text{duration}] + 1.14$, $r^2 = 0.28$, $P = 0.003$). I found no significant differences across biomes, fertilizer types or methods of measurement (Table 1). Within biomes, N effects were significantly positive in deserts and significantly negative in boreal forests. Studies that measured microbial biomass via chloroform fumigation were associated with a significant decline of 18%. Microbial response ratio was not significantly related to fertilization rate (data not shown, $P = 0.238$) or N deposition rate (data not shown, $P = 0.774$).

Fungi

Across all studies, fungal responses to N were not significant; response ratios ranged between 0.770 and 1.188 (95% CI; Table 1). In addition, fungi studies were not significantly heterogeneous in terms of response ratio ($Q_T = 16.15$, $P = 0.372$). However, fungal biomass declined more strongly as the total load and duration of N fertilization increased (Fig. 2). Background rates of N deposition were not significantly related to response ratios (data not shown, $P = 0.336$). Response ratios were significantly greater under higher N fertilization rates (Fig. 2). However, the study by Tingey *et al.* (1997) was associated with a particularly high N fertilization rate (200 kg N ha^{-1} $year^{-1}$) and a high *R* (2.0), and it had a large leverage (0.370). When this study was removed from the analysis, N fertilization rate did not have a significant effect ($r^2 = 0.14$, $P = 0.270$). There were no significant differences among biomes, fertilizer types or units of measurement (Table 1). Woodland/shrubland was the only biome to display a significant N effect, with a 9% decline in fungal response ratio.

Bacteria

As a whole, studies of bacterial biomass did not indicate a significant change under N fertilization (Table 1). Responses were not significantly heterogeneous among studies ($Q_T = 6.34$, $P = 0.785$), and the 95% CI of *R* ranged from 0.928 to 1.133. No significant effects of biome, measurement assay, fertilization rate ($P = 0.656$), N load ($P = 0.602$), background N deposition rate ($P = 0.524$) or study duration ($P = 0.714$) were evident (data not shown). Within biomes, bacterial response ratios increased significantly in boreal forests and decreased significantly in tundra.

Microbe, fungi and bacteria studies combined

Across all microbe, fungi and bacteria studies, response ratios did not vary significantly with replicate number (data not shown, $P = 0.664$). Plot size was negatively related to response ratio ($\ln R = -0.0003 * \text{size} + 0.059$, $P = 0.011$), possibly because studies with larger plot sizes tended to be carried out for longer durations ($r = 0.492$, $P < 0.001$).

The Kendall's tau rank correlation test for publication bias was marginally significant ($\tau = 0.140$, $P = 0.064$), indicating that stronger N effects were somewhat more likely to be published than were weaker N effects.

Soil CO_2 emissions

Seventeen studies reported N effects on soil CO_2 emissions as well as N effects on microbial, fungal or bacterial biomass. Across these studies, response ratios of biomass were significantly and positively correlated with those of soil CO_2 respiration (Fig. 3).

DISCUSSION

For the microbial community as a whole, biomass declined by an average of 15% under N fertilization. Moreover, biomass decreased more substantially under larger N loads

Table 1 Results of statistical comparisons among and within groups

Comparison	Group	*R*	95% CI	Number of studies	Q_M	Q_E	*P*-value among groups†
Microbes	All microbe studies*	0.846	0.700–0.977	29			
Biome	Agricultural	0.698	0.360–1.050	7	7.50	35.38	0.264
	Boreal forest*	0.782	0.628–0.900	4			
	Desert*	1.496	1.128–1.842	2			
	Temperate forest	0.796	0.578–1.004	10			
	Temperate grassland	1.038	0.998–1.072	4			
Fertilizer type	NH_4NO_3	0.895	0.753–1.082	15	5.34	23.19	0.393
	$NaNO_3$	1.010	0.814–1.235	5			
	NH_4Cl	0.608	0.305–1.077	2			
	$(NH_4)_2SO_4$*	0.727	0.620–0.890	2			
	$Ca(NO_3)_2$*	1.184	1.035–1.360	2			
Measurement	Chloroform fumigation*	0.823	0.654–0.985	24	0.17	36.79	0.723
	PLFA	0.903	0.685–1.165	4			
Fungi	All fungi studies	0.977	0.770–1.188	16			
Biome	Boreal forest	0.717	0.402–1.434	4	2.33	19.06	0.383
	Temperate forest	0.991	0.716–1.347	7			
	Woodland/Shrubland*	1.092	1.074–1.108	3			
Fertilizer type	NH_4NO_3	0.949	0.732–1.159	11	0.027	10.75	0.960
	$NaNO_3$	1.010	0.968–1.050	2			
	$(NH_4)_2SO_4$	0.965	0.452–2.000	2			
Measurement	Microscopy	1.070	0.949–1.229	5	4.42	10.98	0.173
	PLFA	0.759	0.480–1.046	6			
	Minirhizotron	1.334	0.946–1.841	5			
Bacteria	All bacteria studies	1.028	0.928–1.133	11			
Biome	Boreal forest*	1.061	1.050–1.062	2	1.61	3.48	0.558
	Temperate forest	0.959	0.858–1.080	5			
	Tundra*	0.779	0.705–0.860	2			
Measurement	Microscopy	0.969	0.840–1.109	7	0.38	5.27	0.518
	PLFA*	1.053	1.040–1.062	2			

PLFA, phospholipid fatty acid.
*Significant N effect on group ($P < 0.05$).
†Only groups represented by two or more studies were included in comparisons.

and longer durations of fertilization. These results supported my first hypothesis. Field studies have long reported negative effects of N fertilization (Baath *et al.* 1981; Soderstrom *et al.* 1983), but wide variation in results among systems has hindered our ability to discern general patterns qualitatively (Fog 1988). Older studies primarily used chloroform fumigation to measure microbial biomass. This technique, in particular, was associated with a significant negative response in my meta-analysis. PLFA-derived data in more recent studies were not significantly different from chloroform fumigation-derived data, which indicates that the two methods yielded fairly consistent results.

Fungi also became scarcer as N load and duration increased. Across all fungal studies, though, I observed no significant decline with N fertilization, owing to the presence of a number of studies reporting positive effects of N. Positive effects tended to be more frequent among studies with lower N loads and shorter durations. As methods for measuring microbial biomass should have included fungal biomass, it is likely that fungal responses contributed to the microbial decline under high N loads and longer study duration. The negative relationships between fungal biomass and N load and duration may be attributable to a progressive inhibition of growth or ligninase activity of white rot fungi (Ander & Eriksson 1977; Keyser *et al.* 1978; Waldrop & Zak 2006), or to condensation of organic compounds with N-containing compounds (Soderstrom *et al.* 1983; Fog 1988). High N availability may also induce 'browning' (i.e. production of brown, recalcitrant compounds) of plant material, leading to the accumulation of

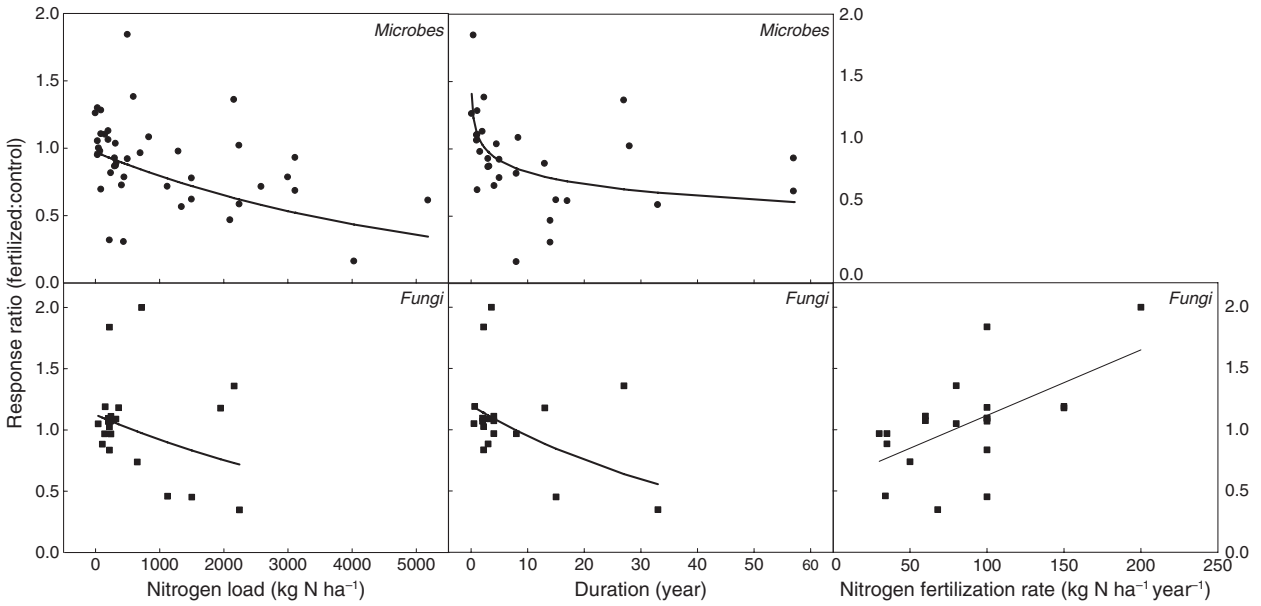

Figure 2 Response ratios (fertilized : control) of microbial and fungal biomass as a function of N load and duration of fertilization. Nitrogen load represents the total amount of N added over the course of the experiment. Line is the best-fit regression. Each symbol represents one study. Circles – microbiol biomass, squares – fungal biomass. Response ratio of bacterial biomass was not significantly related to N load or duration, so these data are not shown. Response ratios of microbes and fungi declined significantly with increasing duration (microbes: $R = -0.13 * \ln[\text{duration}] + 1.12$, $r^2 = 0.25$, $P = 0.003$; fungi: $\ln R = -0.025 *$ duration $+ 0.18$, $r^2 = 0.28$, $P = 0.049$) and N load (microbes: $\ln R = -0.00015 *$ N load $- 0.030$, $r^2 = 0.18$, $P = 0.005$; fungi: $\ln R = -0.0002 *$ N load $+ 0.12$, $r^2 = 0.17$, $P = 0.034$). Conversely, N fertilization rate was positively associated with fungal response ratio ($\ln R = 0.0049 *$ N fertilization rate $- 0.47$; $r^2 = 0.34$, $P = 0.014$).

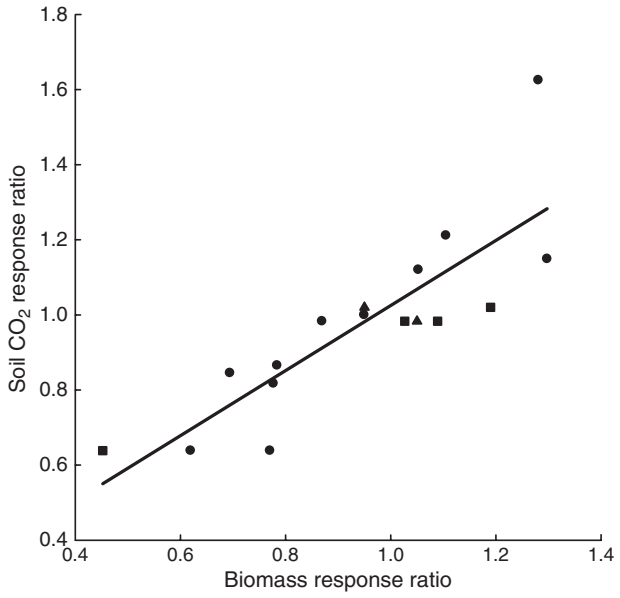

Figure 3 Relationship between response ratios (fertilized : control) of soil CO_2 emissions and response ratios of biomass (microbial, fungal and bacterial). Line is best-fit regression through all points (CO_2 $R = 0.87 *$ microbial $R + 0.16$, $r^2 = 0.836$, $P < 0.001$). Each symbol designates one study. Circles – microbial biomass, squares – fungal biomass, triangles – bacterial biomass.

compounds that are toxic to fungi (Hodge 1953; Haider & Martin 1967; Soderstrom *et al.* 1983; Fog 1988).

Mycorrhizal fungi might have been adversely affected by N fertilization, given that plants tend to allocate less C to mycorrhizal fungi when N is more available (Read 1991; Treseder 2004). However, plant-free microcosms have also yielded decreases in fungal biomass under N additions (Bollen & Lu 1961; Mangenot & Reymond 1963). In these cases, mycorrhizal fungi should have been relatively dormant in control and N-enriched samples alike, because no host plants were present. In addition, declines in fungal biomass were not evident in boreal or temperate forests or woodland/shrublands in the meta-analysis, even though these biomes tend to be dominated by ectomycorrhizal fungi (Allen 1991; Vogt *et al.* 1991; Nilsson & Wallander 2003). Inhibition of mycorrhizal fungi may not completely explain the drop in fungal abundance.

Unlike fungi, bacteria did not significantly respond to N fertilization, either overall or as a function of N load, N fertilization rate or study duration. This lack of response is contradictory to my first hypothesis. Bacteria were represented by fewer studies (i.e. 11) than were microbes (29) and fungi (16). The smaller sample size may have limited my ability to detect significant differences. However, the 95% CIs for bacteria response ratio were relatively narrow,

ranging from a 7% decrease to a 13% increase. Moreover, there was no significant heterogeneity among bacterial studies. The 11 studies included in the meta-analysis may have been sufficient to detect general trends.

In addition to changes in biomass, community composition within fungal and bacterial groups can shift under N additions. The past few decades have witnessed a broad decline in the diversity of fungal sporocarps produced in forests exposed to heavy anthropogenic N deposition (Arnolds 1988). Species shifts in ectomycorrhizal fungi, in particular, have received much attention (Wallenda & Kottke 1998; Lilleskov & Bruns 2001). Ectomycorrhizal diversity frequently decreases following N fertilization or deposition (Arnolds 1991; Lilleskov *et al.* 2001, 2002; Peter *et al.* 2001; Frey *et al.* 2004), although belowground changes tend to be less pronounced than aboveground changes (Karen & Nylund 1997; Jonsson *et al.* 2000; Peter *et al.* 2001). Nitrogen fertilization has also reduced the taxa richness of decomposer fungi belowground in Alaskan boreal forest (Allison *et al.* 2007), potentially owing to the inhibition of wood-decomposing fungi. Changes in community composition under N additions have been noted in bacteria as well. Among ammonia oxidizers, species in *Nitrosospira* 16S cluster 3 tend to become more dominant (Bruns *et al.* 1999; Horz *et al.* 2004). Fatty acid methyl ester and PLFA profiles have been used to detect modest changes in major bacterial and fungal groups in temperate forests (Gallo *et al.* 2004; Nilsson *et al.* 2007) and in coastal sage scrub (Siguenza *et al.* 2006). These alterations in microbial communities indicate that certain taxa respond more strongly to N than do others. Species shifts might accentuate or mitigate ecosystem-level effects of alterations in biomass, depending on the functional roles of individual taxa.

Net primary productivity of plants is frequently limited by N (Vitousek & Howarth 1991; LeBauer & Treseder 2008). One might expect that fungi and bacteria would likewise be N-limited, given their relatively high tissue N concentrations coupled with high growth rates and the production of N-rich extracellular enzymes (Paustian & Schnurer 1987; Paul & Clark 1996; Fenchel *et al.* 1998; Dighton 2003). Each trait should contribute to relatively high demands for N, which would induce N-limitation if N supplies were insufficient. Yet, no target group displayed a significant increase in biomass under N fertilization. Nitrogen limitation did not appear to be widespread among microbes.

Another consideration is that the standing biomass of microbes does not necessarily reflect growth rate. Growth and death rates could have both increased even while standing biomass dropped. Few studies have directly measured these rates in the soil under N fertilization. Stapleton *et al.* (2005) used thymidine incorporation to assess bacterial growth, and they observed positive effects of N additions although standing biomass did not change. Using the same approach, Demoling *et al.* (2008) found declines in bacterial growth in two of three field sites. In the same study, fungal growth rates were determined by measuring rates of acetate incorporation into ergosterol; these rates did not change under N fertilization. In a minirhizotron-based system, lifespans of fungal rhizomorphs were unaltered by N (Treseder *et al.* 2005). If measurements of growth rates and lifespans become more common, we can then better determine general patterns of microbial productivity.

For longer-term studies, an increase in aboveground litter production by plants could have alleviated C limitation of microbes. However, this reaction was not evident for any target group, as response ratios declined or remained steady with increasing study duration. Microbial biomass might instead be limited by the availability of water, phosphorus or belowground C sources (Baath *et al.* 1981; Biederbeck *et al.* 1984; Scheu 1990; Vance & Chapin 2001; Demoling *et al.* 2007).

The moderate decrease in microbial biomass under N fertilization may have consequences for C fluxes. Soil CO_2 emissions declined in concert with declines in microbial biomass, which supports my second hypothesis. Schimel & Weintraub (2003) constructed an enzyme kinetics-based model that predicted N-limited microbes could display a decrease in CO_2 respiration after N fertilization, as added N would stimulate the incorporation of C into new tissues instead. This prediction was not well-supported by the studies represented here – soil CO_2 efflux was positively correlated with microbial biomass, even when R was >1. In a meta-analysis of N effects on decomposition rates, litter mass loss tended to decline under N additions (Knorr *et al.* 2005). Fluxes of CO_2 from the soil to the atmosphere may slow as N availability increases, with a reduction in the soil microbial biomass and activity as the underlying mechanism. As negative effects on microbial biomass were more common where fertilization lasted longer than 5 years, corresponding declines in soil CO_2 release may also be more likely after longer-term manipulations.

Contrary to my hypothesis, ambient levels of N deposition did not appear to mediate biomass responses to N fertilization across studies. In other words, N deposition rates did not significantly influence response ratios of microbes, fungi or bacteria. This broad-scale finding is consistent with that of DeForest *et al.* (2004), who found that northern hardwood forests exposed to wet + dry N deposition ranging from 6.8 to 11.8 kg N ha^{-1} $year^{-1}$ did not vary significantly in the degree to which microbial biomass decreased under N fertilization. In contrast, PLFA analyses conducted by Demoling *et al.* (2008) indicated that actinomycetes represented by the PLFAs 10 Me 16 : 0, 10 Me 17 : 0 and 10 Me 18 : 0 responded differently to N fertilization among three forests exposed to varying levels of ambient N deposition. Other studies have reported declines

in the abundance of actinomycetes (Siguenza *et al.* 2006), ectomycorrhizal fungi (Lilleskov *et al.* 2001; Nilsson *et al.* 2007) and certain gram-negative bacteria (Nilsson *et al.* 2007) along increasing gradients of ambient N deposition. These findings indicate that microbial biomass can be affected by the current levels of anthropogenic N deposition when analyses are constrained to a smaller geographic scale.

There was no evidence for significant effects of N fertilizer type on microbial response ratio across studies. Similarly, in individual comparisons within ecosystems, no differences have been observed between sodium nitrate and ammonium chloride (Lucas *et al.* 2007), between ammonium–N and nitrate–N (Stapleton *et al.* 2005), or between ammonium nitrate and urea (Baath *et al.* 1981; Nohrstedt *et al.* 1989). Although there were no significant differences among fertilizer types, calcium nitrate and ammonium sulphate were each associated with mean response ratios that significantly departed from 1. In particular, microbial biomass increased by 18% under calcium nitrate additions. This increase may be attributable to alleviation of calcium limitation, which can occur in ecosystems exposed to long-term N saturation (e.g. McNulty & Aber 1993). It appears that the chemical composition of the extra N source has a relatively minor effect on microbial responses, although the identity of any associated ions may be important.

Likewise, biomes did not differ significantly from one another in N effects. Only a few biomes were well replicated for any target group, though. Temperate forests, mostly in North America, were the most common biome type. In contrast, no studies of tropical forests and savannas met the criteria required for the meta-analysis. These biomes were not included. Nitrogen effects on microbes have been examined in the tropics, but urea was commonly used as N fertilizer. In addition, phosphorus fertilizer was often applied in combination with N fertilizer. Each of these approaches could have obscured N effects via the addition of other nutrients, so they were omitted from the analysis. Additional N studies in tropical areas are critical, as N and phosphorus availability in these biomes can be quite different from those in other latitudes (Walker & Syers 1976; Reich & Oleksyn 2004).

CONCLUSIONS

In summary, N additions had a negative effect on microbial biomass, and the reduction of microbial and fungal biomass became more evident as N load and duration of fertilization increased. Moreover, soil CO_2 emissions responded in concert with biomass. These results imply that anthropogenic N deposition may lead to a decline in the release of this greenhouse gas from soils to the atmosphere, owing to the inhibition of microbial growth and activity. In addition, this response may become more evident in ecosystems exposed to longer-term and larger amounts of N. Anthropogenic N deposition is also predicted to augment NPP (LeBauer & Treseder 2008), which should increase fluxes of C from the atmosphere into ecosystems. If soil CO_2 emissions decrease as well, the net effect may be a reduction in atmospheric CO_2 concentrations, and possibly an increase in runoff of dissolved organic C from ecosystems.

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